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Detection of drinking water contamination by an optical real-time bacteria sensor

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Abstract

In a drinking water distribution system, little is known about the characteristics of a microbiological pollution, how it enters the system, and how it can be detected. The drinking water industry has relied on various pollution indicators, through grab sampling and laboratory analyses, revealing results long after the water has been used. To be able to react more proactively to pollution events, many drinking water distributors supplement grab sampling with proportional sampling and/or real-time sensors. We have tested the ability of a new bacteria monitor to detect four different pollution events: wastewater intrusion, rainwater runoff, resuspension of drinking water sediments, and bird droppings entering the distribution system. The monitor response, in terms of bacteria and abiotic particle concentrations, was compared with traditional laboratory methods. The results illustrate the benefits of using such real-time bacteria sensors for monitoring the dynamics of drinking water microbiology and for early warning of potential pollution events.

Key words: bacteria monitor, drinking water, early warning, real-time sensor, pollution detection

INTRODUCTION

Clean healthy drinking water is commonly characterized as water that complies with local regulation. In most cases, monitoring of water quality is carried out through grab sampling, representing individual points in time and space, combined with laborious laboratory analyses yielding results within days. The probability of catching a periodic pollution event is thus very small (Besmer & Hammes 2016) and the chance of acting on it even smaller.

What utilities lack to be able to react proactively on pollution events is real-time monitoring methods with a high timely resolution going beyond current legislations. Fast methods measuring, e.g. conductivity, oxygen level, pH, and turbidity, are already implemented in many systems, and they are continuously becoming more robust and maintenance free (Raich 2013; Banna *et al.* 2014). However, they do not measure changes in microbiological load, but merely provide indicators that may or may not correlate with the number and activity of microorganisms. Methods that target

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microorganisms more directly do exist, though they are mostly laboratory-based methods or require handling of samples prior to analysis (Lopez-Roldan *et al.* 2013). Some of the most recent methods that have been tested in the field include flow cytometry (Prest *et al.* 2013), ATP (Vang *et al.* 2014), and optical bacteria detection (Højris *et al.* 2016). Both flow cytometry and ATP monitoring require sensitive chemicals, create waste, and require regular maintenance. The latter optical technology is, on the other hand, chemical free and requires little maintenance. It was recently demonstrated in laboratory and field tests to be able to monitor the concentration of bacteria and abiotic particles with a 10-minute resolution (Højris *et al.* 2016).

We have tested the ability of this optical bacteria monitor to detect different types of potential drinking water pollutants and compared this ability to that of traditional laboratory-based methods and turbidity measurements. As model pollutants, we have applied municipal wastewater, surface water runoff/soil, distribution system sediments, and bird excrement. Unintended cross-connection between drinking water and wastewater systems (Vestergaard *et al.* 2007) and bird excrement from partly open storage facilities, have each been associated with at least one major pollution event in Denmark, and surface water runoff is known to cause periodic events of elevated plate counts and possibly presence of *E. coli* during the shift from dry to wet periods (Kistemann *et al.* 2002). Sediments in drinking water distribution systems may be loosened during pressure shocks and could influence monitoring of both bacteria and abiotic particles (Christensen 2011).

METHODS

The applied sensor utilizes three-dimensional image analysis of individual suspended particles, including bacteria, and reports the identified objects as either bacteria or abiotic particles. It analyses batches of water samples with a time resolution of 10 minutes. A detailed description of the technology has been published elsewhere (Højris *et al.* 2016).

Laboratory tests of the optical sensor were carried out at Technical University of Denmark, Department of Environmental Engineering, Kgs. Lyngby, Denmark (DTU), and Grundfos Holding A/S, Bjerringbro, Denmark (Grundfos). The GRUNDFOS BACMON bacteria monitor (hereafter named the sensor) and accompanying exchangeable flow cells were supplied by Grundfos Holding A/S, Denmark.

At DTU, spiking tests were performed using: (a) raw municipal wastewater, (b) surface water runoff sampled from a puddle of water following a rain event, and (c) sediments from a drinking water distribution network sampled from flushing a fire hydrant. At Grundfos, spiking tests were performed using: (a) raw municipal wastewater sampled at Bjerringbro municipal wastewater treatment plant after sand trap and coarse filtration, (b) surface water runoff simulated by soil taken from 10 cm depth, and (c) bird excrement sampled at a pigeon-breeding member of Bjerringbro Poultry Association. Solid samples were suspended in filter-sterilized (0.2 micrometre) tap water, allowed to sediment for 1 hour, and decanted.

The pollutants were added to tap water from the two locations. The two tap water qualities were analysed using the same methods as the spiked samples to establish baseline average and deviation. Total Direct Cell Count (TDCC) was performed by DAPI (4′,6-diamidino-2-phenylindole) staining and epi-fluorescence microscopy (DTU: Olympus BH-2 microscope (Japan), Grundfos: Zeiss Axio Imager 2 (Germany)). Prior to staining, samples containing aggregates were treated with ultrasound (Branson Sonifier 250) for 1 minute to split the aggregates in individual particles and bacteria. Heterotrophic Plate Counts (HPC) were carried out by pour plate in yeast extract agar incubated for either 68 $\pm$ 4 hours at 22 °C or 44 $\pm$ 4 hours at 37 °C. ATP measurements were performed on an Advance Coupe luminometer (Celsis) with Lumin(ATE)/Lumin(EX) reagents (Celsis). Turbidity measurements

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were carried out on a Turb 430 IR field turbidity meter (Wissenschaftlich Technische Werkstätten – WTW) (at DTU) or a Turbiquant 3000 IR turbidimeter (HACH) (at Grundfos). Conductivity measurements were carried out on a Cond 3310 SET 1 field equipment (WTW), or on a CDM 83 Conductivity Meter (Radiometer Copenhagen) (both at DTU).

RESULTS AND DISCUSSION

Spiking tap water with three potential pollution sources, surface water runoff, domestic waste water and pigeon faeces, showed at which concentrations the pollution could be detected (Figure 1). For each series of measurements, including sensor responses, turbidity, and total direct cell counts (DAPI), the average and standard deviations of spiked samples were compared to the average and standard deviation of the tap water itself.

Applying the student T-test ($\alpha = 0.015$) to the spikes and background measurements, gave the levels at which the added pollution source could be significantly detected. The results of the T-tests are shown in Figure 2. For surface water runoff, the abiotic particle variable detected the pollution at 2 mg/L dry soil, total cell counts at 6.7 mg/L and turbidity and the bacteria variable both at 20 mg/L. For wastewater, the abiotic particle variable detected the pollution at 0.1 mg/L dry soil, total cell counts and the bacteria variable both at 0.3 mg/L and turbidity at 3 mg/L. For pigeon faeces, all except turbidity detected the pollution at 0.5 mg/L dry faeces, whereas turbidity only detected it at 5 mg/L.

Spiking tests performed at DTU (see Figure 3) all significantly differed from the background (T-test, $\alpha = 0.05$). Background levels in the un-spiked tap water were: Bacteria (sensor) 6,440 $\pm$ 1,330 mL^{-1}, Abiotic particles 22,000 $\pm$ 2,060, HPC 37 °C 9.9 $\pm$ 1.7 CFU/mL, HPC 22 °C 9.9 $\pm$ 1.7 CFU/mL, Turbidity 0.2654 $\pm$ 0.04 NTU, ATP 1.81 $\pm$ 0.24 pg ATP/mL (average $\pm$ one standard deviation). In all spiking tests, a good linearity ($R^2 > 0.975$) was observed for all parameters measured.

To address the accuracy of single measurements, a series of tests was made at DTU in which the water sample was kept stagnant within the flow cell during several measurements (data not shown). The lower values measured for bacteria in tap water samples expressed a higher deviation (15–30%) compared to bacteria in spiked samples (9–14%) and abiotic particles in all samples (4–13%).

The total number of particles detected by the sensor (the sum of bacteria and abiotic particles) is expected to corelate with the turbidity of the water for non-coloured samples. The lower threshold of the sensor in terms of particle size does, however, leave out particles smaller than approximately 500 nm, which to some extent limits this correlation (Højris *et al.* 2016). Further, as the turbidity contribution is different for different particle sizes and diffractive indexes (Melik & Folger 1983; Cheng *et al.* 2010), the correlation may be further compromised.

Comparing the sum of bacteria and abiotic particles measured by the sensor to turbidity for each individual pollution simulation (see Figure 4) shows good correlation between the total concentration of particles (bacteria + abiotic particles) and turbidity. However, the slope differs significantly between the different pollution sources. The two tests simulating pollution from surface water runoff, carried out at two different locations and analysed at two different laboratories, have similar slopes, whereas the two tests using municipal wastewater show different slopes. This observation is in good agreement with previous studies (McCoy & Olson 1986).

The number of bacteria measured by the sensor is also expected to corelate with ATP, given the cells involved have similar amounts of ATP. Comparing bacteria measured by the sensor and ATP, for the two tests where ATP was measured (see Figure 5), shows a good correlation, which in the two cases is independent on the type of pollution. However, it may be expected that bacterial cells from other more nutrient rich pollution sources, like faeces, have a higher ATP content and that the slope in such cases are different.

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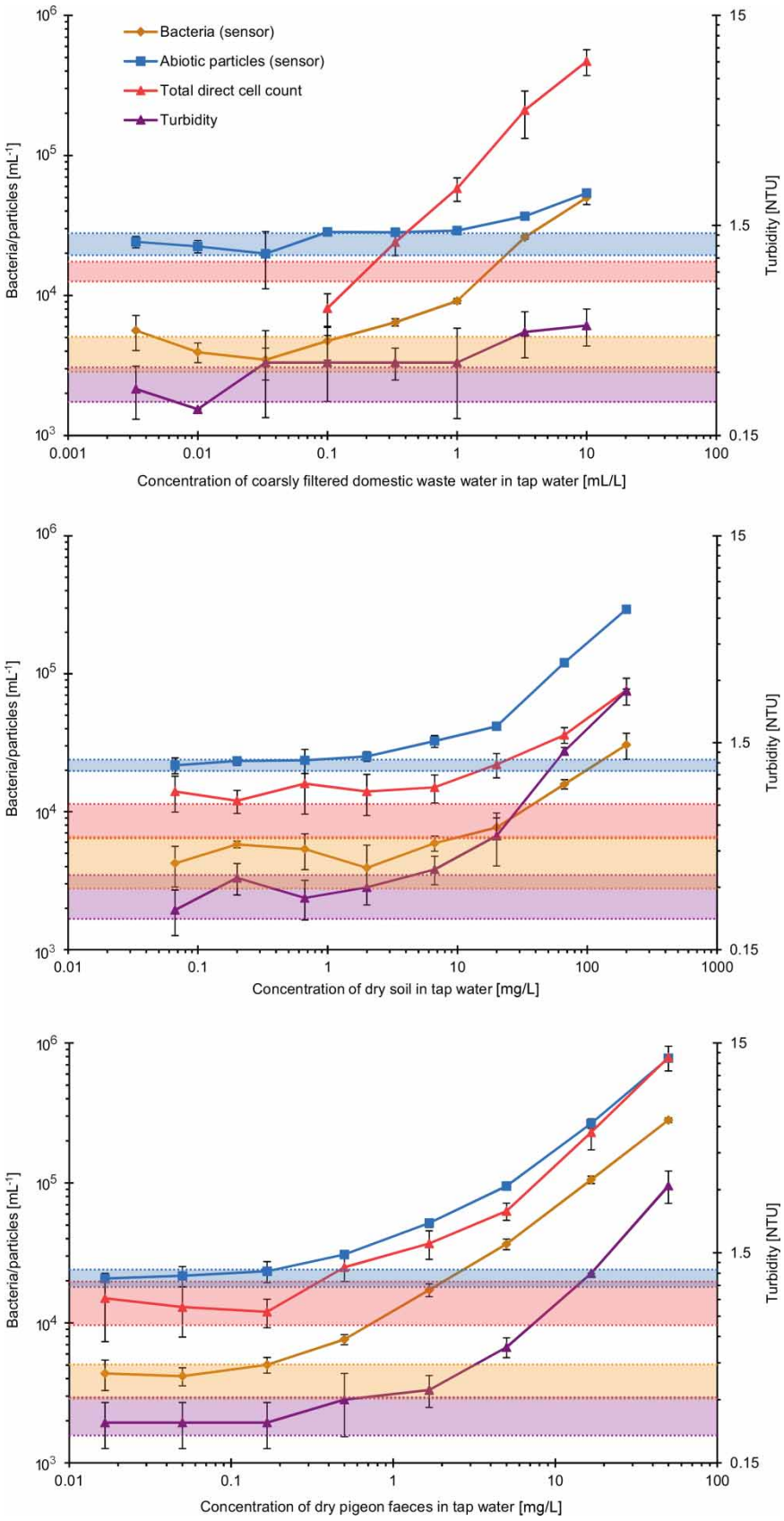

Figure 1 | Results from spiking drinking water (at Grundfos) with three potential pollution sources: domestic waste water (top/right), surface water runoff simulated by soil suspension (middle), and pigeon faeces (bottom/left). Error bars indicate ± one standard deviation. Horizontal bands represent background levels spanned by averages ± one standard deviation.

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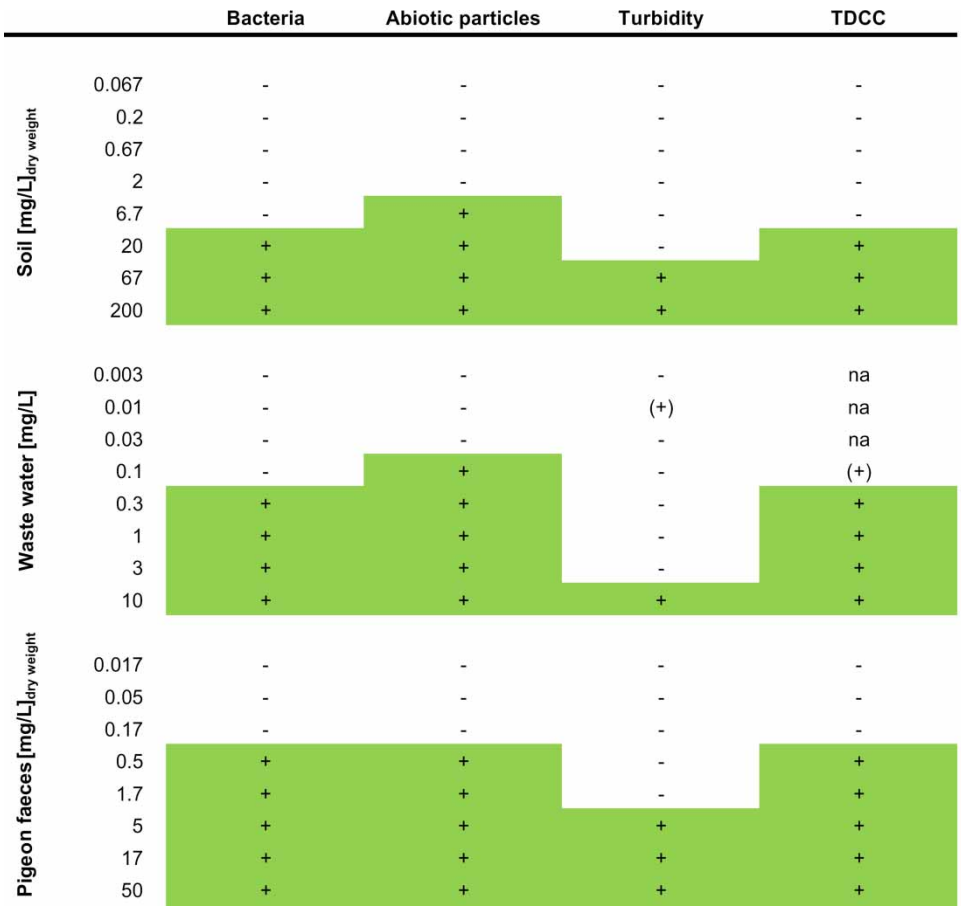

Figure 2 | Significant deviation from background levels based on students T-test ($\alpha = 0.015$). '+' indicates when the responses of the different methods are significantly higher than the background. '(+)' indicates responses significantly lower than the background. 'na' indicates that analysis was not possible. The bacteria and abiotic particles are measured by the sensor and it is seen that it reacts 10–100 times before the turbidity meter and at the same contamination levels as total direct cell counts (TDCC).

Assuming HPC reveal relevant fractions of the total populations, and that these fractions are comparable, some correlation could be expected between the bacteria measured by the sensor and plate counts. Comparing the results from the two tests where both parameters were measured (see Figure 6) does show a relatively good correlation. The fraction of the total cell number as measured by the sensor that can be identified with HPC is, however, different depending on the pollution level. As the measured plate counts spans four decades, the total number of bacteria as measured by the sensor only spans two. Clearly, there are different fractions of bacteria that are not detected by the plate count method, which is also the result of several previous studies (Byrd *et al.* 1991; Allen *et al.* 2004; Chowdhury 2012).

As published earlier (Højris *et al.* 2016), there is a relatively good correlation between the number of bacteria detected by the sensor and the TDCC performed manually on a microscope. The results of the presented tests support this as there is close to a 1:1 relationship between the two measures (see Figure 7). In the test simulating surface water runoff performed at Grundfos, the total direct cell counts were, however, higher than the numbers reported by the sensor, still with a good linear correlation. A plausible explanation could be that the cells in the suspended soil used for the test were clustered and that the clusters were broken up prior to analysing the TDCC analyses.

Using plate counts as a tool to identify pollution of drinking water has been practiced for many years, regardless the increasing acceptance that the method only reveals an unknown fraction of the entire population (Byrd *et al.* 1991; Allen *et al.* 2004; Chowdhury 2012). In the two tests

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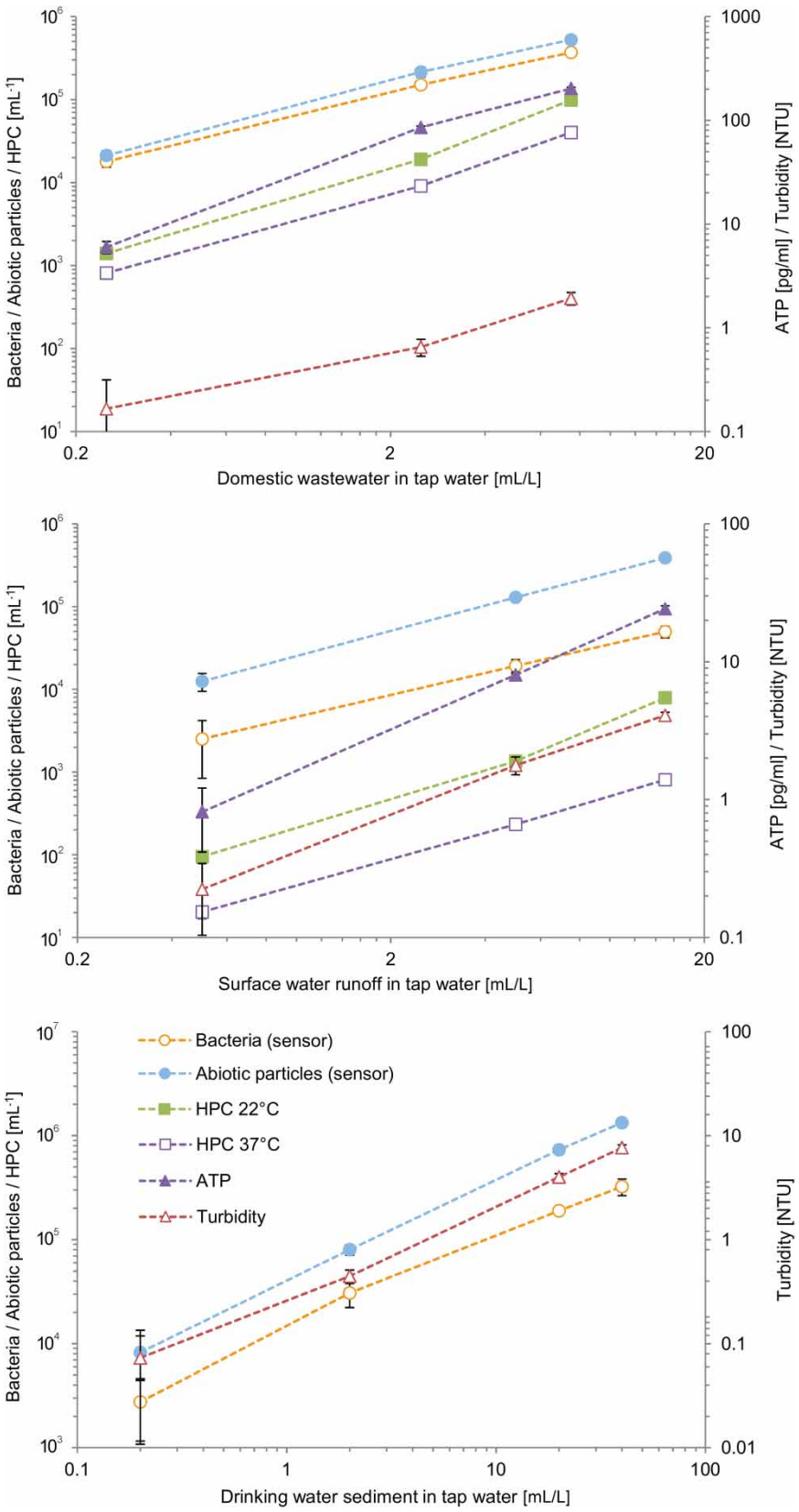

Figure 3 | Results from spiking drinking water (at DTU) with three potential pollution sources: domestic waste water (top), surface water runoff simulated by puddle water (middle), and sediment from a drinking water distribution system (bottom). Error bars indicate ± one standard deviation. Background levels have been subtracted and combined standard deviations calculated.

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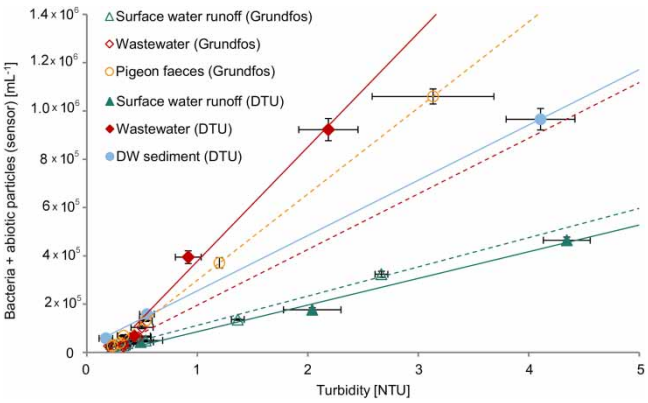

Figure 4 | Total particle concentration as the sum of bacteria and abiotic particle (both measured by the sensor) plotted against turbidity for each spiking test. The total particle concentration correlates well with turbidity within each test, but the coefficient differs among the different pollution sources. The two surface water run-off simulations express the same coefficient, whereas the two waste water simulations are different.

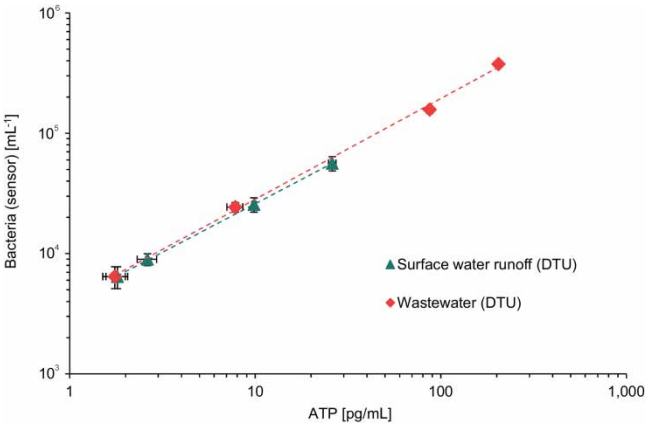

Figure 5 | Bacteria concentration (measured by the sensor) plotted against ATP for wastewater and surface water run-off (both performed at DTU). In both tests a good correlation was found between the bacteria and ATP concentrations. Note that the regression lines of the two different pollution sources coincide.

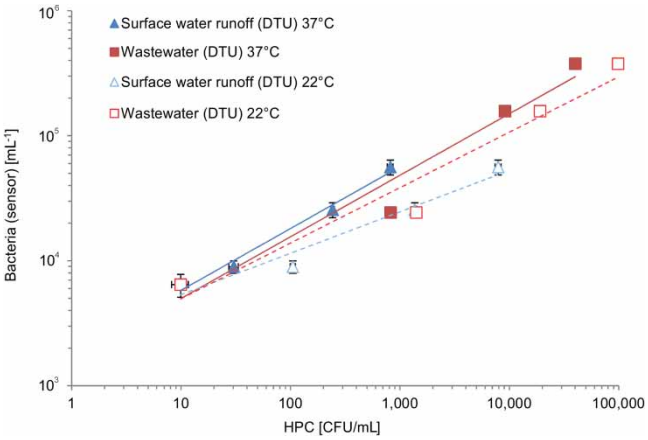

Figure 6 | Bacteria concentration (measured by the sensor) plotted against heterotrophic plate counts (HPC) at 22 and 37 °C for wastewater and surface water run-off (both performed at DTU).

shown in Figure 6, one colony on a plate may thus represent a number of bacteria between four and 650. Similar observations may be made regarding the use of turbidity as an approximate or indirect measure of bacterial pollution. Though a good correlation has been shown for each pollution

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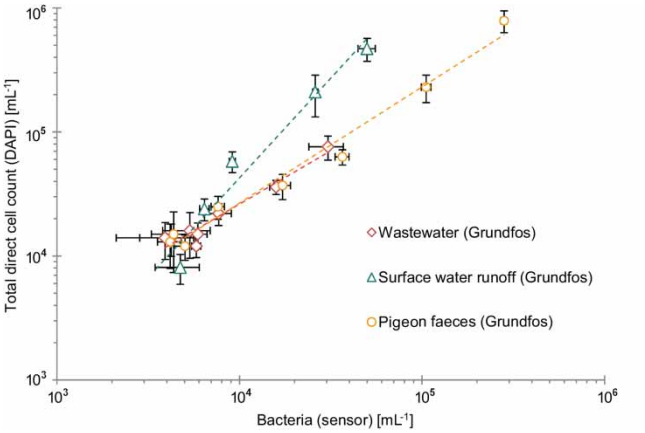

Figure 7 | Bacteria concentration (measured by the sensor) plotted against total direct cell counts (DAPI) for wastewater, surface water run-off, and pigeon faeces (all performed at Grundfos).

source, it is not possible to estimate the number of bacteria and abiotic particles based on a turbidity measurement, unless the type of pollution and the corresponding correlation is known beforehand.

TDCC or number of bacteria as measured by the sensor seems to be a more precise measure of bacterial pollution of drinking water. With the several days' turnover for plate counts or the laborious manual microscopy of the total direct count, the sensor's time resolution of 10 minutes approaches a real-time measure. Though the sensor does not count the individual cells if these are clustered, it gives a better approximation of the total cell number than both plate counts and turbidity.

CONCLUSIONS

The results show a good correlation between the sensor's bacteria signal and total direct cell counts performed in the laboratory. The sensor's ability to detect different simulated pollution events was shown to be comparable to laboratory-based total direct cell counts and the concentrations at which the pollution was detected was one half to two decades lower than the detection limit for turbidity. A good correlation was seen against ATP, but the correlation with turbidity (against the sum of bacteria and abiotic particles from the sensor) depended upon the pollution source. The applied sensor and plate counts showed similar responses, but the ratio between the two ranged from 4:1 in the most polluted samples to 650:1 in the diluted ones. These results demonstrate the benefit of the sensor over turbidity sensors in monitoring bacteria in drinking water, detecting potential pollution events, and illustrate the applicability of the bacteria sensor as early warning of microbiological pollution of drinking water.

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